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REMARKS/ARGUMENTS

Claims 20-29 are pending in the present application. Claims 1-19 are canceled without prejudice. Applicants reserve the right to file a divisional application to prosecute claims 1-19. Claim 29 is newly added, which further makes clear that the dye in the originally recited "staining solution" of "a reagent for staining bacteria" is "capable of staining bacteria". Support for the amendment can be found, at, for example, page 10, line 13-page 14, line 16. No new matter is entered in the present amendment. In fact, it is Applicants' opinion that a person of ordinary skill in the art would understand that the dye in the previously presented claim 20 is capable of staining bacteria, given the context of claim 20, although claim 20 does not explicitly recite so. Hence, the addition of claim 29 would not incur any consideration more than cursory review from the Examiner. Entry of claim 29 is respectfully requested. It is respectfully requested that the Examiner reconsider the rejections based on the following remarks.

Claim Listing

The Examiner pointed out that Applicants' previous amendment did not comply with the new Claim Listing rule under 37 C.F.R. 1.121 (c). In response, this defect has been obviated in the present amendment.

Election/Restriction

As the result of an earlier restriction requirement, non-elected claims 1-19 has been canceled. Applicants retain the right to present claims 1-19 in a divisional application. Claims 20-29 are pending in the present application for the Examiner's consideration.

Anticipation Rejections Under 35 U.S.C. 102

Claims 20, 21, and 25 were rejected as being anticipated by Jackson (U.S. Patent 5,688,011) under 35 U.S.C. 102. Jackson '011 describes a test kit for determining the presence of *helicobacter pylori* using

urease as a marker enzyme. The described kit contains bromophenol blue dye, Tris buffer, and sulfamic acid (col. 2, lines 18-32). The presently pending claim 20 recites a reagent for staining bacteria comprising a staining solution containing a dye; and a diluent containing a buffer for maintaining acidity and an effective amount of a substance capable of reducing nitrite ions. Hence, the Examiner stated that each limitation of claim 20 and 21 of the present invention has been taught by Jackson '011, and therefore rejected claim 20 and 21 as being anticipated by Jackson '011. The Examiner further inferred that since the prior art reagent contains all of the claimed ingredients and can be maintained at the recited pH of claim 25, it is thereby deemed that claim 25 was also anticipated by Jackson '011.

Applicants respectfully traverse. Firstly, **Tris buffer** of Jackson '011 has a buffering effect between **neutral to weakly alkaline**, which is evidenced by, e.g. Exhibit 1 as herein enclosed with the present amendment. Hence, Tris buffer is **not a buffer for maintaining acidity** as recited in claim 20. Under the U.S. patent law, to anticipate a claim, each and every limitation of the claims must be disclosed inherently or expressly by a single prior art reference. Accordingly, none of claims 20, 21 and 25 is anticipated for at least the reason that the limitation of "a buffer for maintaining acidity" is not disclosed by the cited prior art, Jackson '011.

Secondly, the reagent for staining bacteria of claim 20 comprises **a staining solution** containing a dye. As noted above, Jackson '011 determines the presence of *H. Pylori* by detecting the presence of urease because it is well known that the enzyme urease is always associated with H. Pylori. When reacting urea with urease solution, alkaline ammonia is produced. Therefore, the presence of urease can be detected by determining the pH change during the urea-urease reaction (see col.1, lines 37-67). The bromophenol blue dye on the reaction pad of Jackson '011 is used to detect the change of pH, because it changes color when pH changes. The bromophenol blue dye of Jackson '011 serves only to detect a pH change (pH indicator) rather than a dye for staining a bacteria as disclosed by the present invention. Neither the bromophenol blue dye itself nor its combination with any other substance of Jackson '011 is a **staining solution** as recited in claim 20

of the present application. Therefore, this difference constitutes another independent basis that none of claim 20, 21 and 25 is anticipated by Jackson '011.

Obviousness Rejection under 35 U.S.C. 103

Based on the anticipation rejection, the Examiner further rejected claims 20, 21, 24, 25, 27 and 28 under 35 U.S.C. 103(a) as being unpatentable over Jackson '011. Specifically, the Examiner stated that the surfactant, the pH buffer and the dye specifically recited in these claims of the present application would have been known by a person of ordinary skill in the art by routine experimentation or optimization based on the teaching of Jackson '011.

We respectfully traverse. As mentioned above, Jackson '011 does not disclose **a staining solution** nor **the buffer for maintaining acidity** of the present invention. Jackson '011 and the present invention are respectively directed to different applications and uses different mechanisms. Jackson '011 reacts urea with urease solution to produce ammonia and then applies a pH dye to detect the presence of urease to thereby indirectly determine the presence of *H. Pylori* in a gastric biopsy specimen. The present invention, however, uses the staining solution to stain the bacteria, and thereby directly detecting the presence or amount of bacteria by observing, e.g. the intensity of fluorescence emitted from the stained bacteria.

Jackson '011 does not teach that its reagent for detecting presence of *H. Pylori* in a gastric biopsy specimen can be modified by changing the alkaline pH buffer to acidic buffer, and replacing the pH dye with a staining dye, to make a reagent for detecting a bacteria in a sample, particularly a urine sample, as claimed in the present invention.

Nor is there any motivation for a person of ordinary skill in the art to modify Jackson '011 to make a reagent as claimed in the present invention that uses **a different mechanism** from Jackson '011. For example, the bromophenol blue dye of Jackson '011 is used to detect a pH change. A person of ordinary skill in the art

would not be motivated to replace the bromophenol blue dye of Jackson '011 with a dye in a **staining solution** that can stain bacteria and be used for **a different purpose** from the bromophenol blue dye (**pH indicator**).

Moreover, even if a person of ordinary skill in the art would try to modify the reagent of Jackson '011, he or she cannot find any direction in Jackson '011 as to how to proceed with the modification proposed by the Examiner, which option would be successful, which substance is critical, particularly given the fact that the reagent of Jackson '011 uses a different mechanism from the present invention.

As clearly stated by MPEP 2143, to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

As discussed above, in the present case, none of these three criteria is met by the Examiner's rejection, although each of these three criteria must be met to satisfy a proper *prima facie* case of obviousness rejection. Hence, the Examiner's rejection is based on impermissible hindsight at most. Further, if the Examiner is not convinced by the above remarks and insists that a person of ordinary skill in the art would have known how to modify Jackson '011 to arrive at the present invention through routine experimentation, **we request that the Examiner provide us with the evidence supporting such an assertion according to MPEP 2144.03**. The Examiner should inform us of the teachings in the prior art of each limitation of the claims of the present application, and the motivation to combine those teachings, and the expectation of success of the proposed combination.

The Examiner also rejected claims 20-28 under 35 U.S.C. 103(a) as being unpatentable over Jackson '011 and further in view of Jackson (U.S. patent 5,349,801 "Jackson '801"). Specifically, the Examiner applied

the same reasoning as discussed above to reject claims 20, 21, 24, 25, 27 and 28 as being obvious over Jackson '011. Jackson '801 is used to reject claims 22, 23 and 26 because Jackson '801 discloses the use of N-octyl glucose surfactant. Hence, we traverse the Examiner's rejection of claims 20, 21, 24, 25, 27 and 28 for the same reason fully discussed above. As to claims 22, 23 and 26, we traverse as follows.

As noted above, Jackson '011 fails to disclose the **staining solution**, and **a buffer for maintaining acidity** as recited in the claims of the present application. Neither does Jackson '801 teach any of these two limitations. Hence, even if a person of ordinary skill in art had combined the teachings of Jackson '011 and Jackson '801, he or she would still not have obtained the claimed reagent of the present invention.

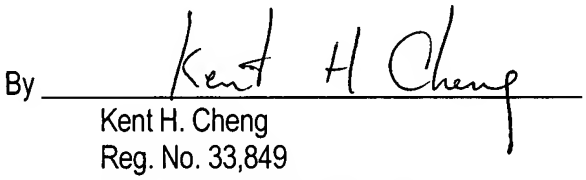
Further, as to the surfactant taught in Jackson '801, the Examiner acknowledged that it is different from the cationic surfactant recited in the claims of the present invention, but stated that selection of the cationic surfactant based on Jackson '801 would have been well within the purview of the artisan of ordinary skill in the art. The Examiner failed to inform us what was the motivation to make the proposed modification, and why a person of ordinary skill in art would expect success with such a proposed modification. Hence, the Examiner failed to establish a *prima facie* case of obviousness.

In contrast with the Examiner's assertion, we believe that a person of ordinary skill in the art would not have made the modification as the Examiner proposed. Similar to Jackson '011, Jackson '801 is also directed to the detection of *H pylori* in endoscopically obtained biopsy specimen by utilizing the reaction between urea and urease, and detecting the pH change caused by the reaction. The N-octyl glucose surfactant of Jackson '801 is employed to aid in freeing the urease enzyme from the biopsy specimen (see col. 4, lines 44-46). On the other hand, the cationic surfactant of the present invention is used not only to improve the stability of bacterial but also dissolve/shrink mucous fibers, erythrocytes, cell fractures etc. which are present in a sample and thereby reduce the effect on detection of bacteria, as described at page 7, lines 5-10, of the present invention. **Hence, a person of ordinary skill in the art would not have modified the surfactant of Jackson '801 to address a problem or attain an object that is different from that of Jackson '801**.

The newly added claim 29 is essentially the same as claim 20, except that claim 29 explicitly requires that the dye be capable of staining bacteria. Hence, the newly added claim 29 is neither anticipated by nor obvious over any prior art reference cited by the Examiner for at least the same reason as discussed in connection with claim 20.

It is believed that no fees or charges are required at this time in connection with the present application; however, if any fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,
COHEN, PONTANI, LIEBERMAN & PAVANE

By 

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Dated: March 11, 2004

Encl. (Exhibit 1)

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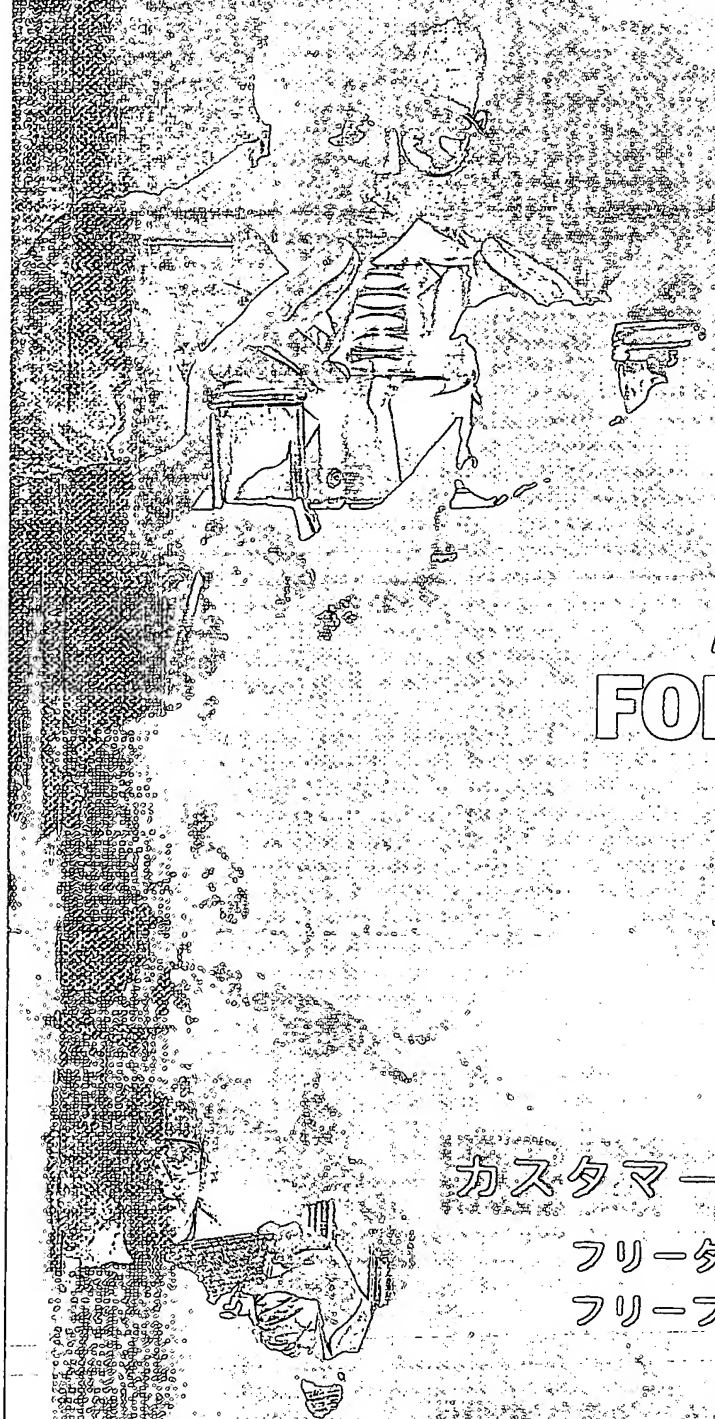
50 YEARS OF SCIENCE, TECHNOLOGY & SERVICE

SIGMA



BIOCHEMICALS AND REAGENTS FOR LIFE SCIENCE RESEARCH

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テクニカルサービス

フリーダイアル ☎ 0120-07-0406

フリーファックス ☎ 0120-67-6788

ALPHABETICAL LIST OF COMPOUNDS

T

TRIZMA®

[Tris(hydroxymethyl)aminomethane]

[Tris; THAM; 2-Amino-2-(hydroxymethyl)-1,3-propanediol; Tromethamine; Trometamol]

- Thoroughly established as an excellent biochemical buffer and basimetric standard.
- Does not precipitate calcium salts. Also of value in maintaining solubility of manganese salts.
- Sigma is the pioneer in Tris for laboratory use. The world's most complete stock of Tris compounds.
- McFarland & Norris report on the use of "Sigma 7-9" for greatly reducing mortality of fish in transport.
- See Biological Buffers Section (*Page 1785*) for other buffers of interest.

Tris has a pKa of 8.1 at 25°C. Trizma Base and Trizma HCl can be blended to produce a buffer at any pH between 7 and 9.

The table specifies the amounts of Trizma HCl and Trizma Base required to prepare 0.05 M buffer solutions at various pH values and temperatures. Dissolve the indicated amounts of Trizma HCl and Trizma Base in water, to a final volume of 1 liter.

Trizma HCl and Trizma Base are somewhat hygroscopic at high humidity. For precise work, desiccation before weighing is recommended. Trizma solutions can be autoclaved.

Trizma Pre-Set Crystals (listed at the end of this section) provide a convenient, accurate alternative to the separate weighing of Trizma Base and Trizma HCl.

Request Sigma Technical Bulletin No. 106B for more information on temperature and concentration effects, and on the use of pH electrodes with Trizma buffers.

pH at temperature			g/L for 0.05 M solution	
5°C	25°C	37°C	Trizma® HCl	Trizma® Base
7.76	7.20	6.91	7.02	0.67
7.89	7.30	7.02	6.85	0.80
7.97	7.40	7.12	6.61	0.97
8.07	7.50	7.22	6.35	1.18
8.18	7.60	7.30	6.06	1.39
8.26	7.70	7.40	5.72	1.66
8.37	7.80	7.52	5.32	1.97
8.48	7.90	7.62	4.88	2.30
8.58	8.00	7.71	4.44	2.65
8.68	8.10	7.80	4.02	2.97
8.78	8.20	7.91	3.54	3.34
8.88	8.30	8.01	3.07	3.70
8.98	8.40	8.10	2.64	4.03
9.09	8.50	8.22	2.21	4.36
9.18	8.60	8.31	1.83	4.65
9.28	8.70	8.42	1.50	4.90
9.36	8.80	8.51	1.23	5.13
9.47	8.90	8.62	0.96	5.32
9.56	9.00	8.70	0.76	5.47

PRODUCT NUMBER			¥
	SIGMA 7-9®	100 g	3000
T 1378	(Tris[hydroxymethyl]aminomethane)	250 g	4200
RT	**White crystalline powder with slight yellow cast**	500 g	5000
	Purity: Minimum 99% (titration)	1 kg	8500
	Absorbance $A^{40\%}_{280}$ **(1 cm path; vs. H_2O):** 0.8 (max.)	5 kg	49100
	Water (Karl Fischer): 0.5% (max.)	10 kg	87000
	Heavy metal (as Pb): 5 ppm (max.)	25 kg	194100
	A 40% (w/w) solution is clear, pale yellow.		
	[77-86-1] $C_4H_{11}NO_3$ FW 121.1		

ELECTRODES
For use with Trizma® Buffers
See: Techware Section *Page 2275*

INDICATOR for titrating Trizma
See: 3-(4-Dimethylamino-1-naphthylazo)-4-methoxybenzenesulfonic acid *Page 393*

PRODUCT NUMBER			¥
	TRIZMA BASE		
	(Tris[hydroxymethyl]aminomethane)		
	pKa=8.1 at 25°C; useful pH range 7-9		
	[77-86-1] $C_4H_{11}NO_3$ FW 121.1		
T 6791	**SigmaUltra**	100 g	670
RT	**>99.9% (titration)**	500 g	2100
	pH (1 M in water, 20°C): 10.5-12.0	1 kg	3850
	Loss on drying (110°C): <0.5%		
	Residue on ignition (SO_4, 900°C): <0.01%		
	Solubility (1 M in water, 20°C): complete, colorless		
	Insoluble matter: passes filter test		
	Chloride (Cl): <0.005%		
	Sulfate (SO_4): <0.005%		
	Al: <0.0005%		
	As: <0.0001%		
	Ba: <0.0005%		
	Bi: <0.0005%		
	Ca: <0.001%		
	Cd: <0.0005%		
	Co: <0.0005%		
	Cr: <0.0005%		
	Cu: <0.0005%		
	A_{260} <0.025; A_{280} <0.020 (1 M in water)		
NEW	**ACS Reagent**	100 g	3100
25,285-9	**Assay (dry basis):** 99.8-100.1%	500 g	7900
RT	**Absorbance:** Passes test		
	Water (H_2O): ≤2%		
	Insoluble matter: ≤0.005%		
	Heavy metals (as Pb): ≤5 ppm		
	Iron (Fe): ≤5 ppm		

TRIZMA®

PRODUCT NUMBER			¥
	(Continuation of)		
	TRIZMA BASE		
T 1503	**White crystalline powder**	25 g	3000
RT	**Primary Standard and Buffer**	100 g	3400
	Reagent Grade	250 g	5800
	Minimum 99.9% (titration)	500 g	8400
	$A^{40\%}_{280}$ **(1 cm path vs. H_2O):** 0.05 (max.)	1 kg	15400
		5 kg	60000
	Water (Karl Fischer): 0.2% (max.)	10 kg	109000
	Heavy metals (as Pb): 2 ppm (max.)		
	A 40% (w/w) solution is clear and colorless.		
	See also: Electrophoresis Reagents *Page 1884*		
	Molecular Biology Products *Page 1551*		
	Tissue Culture Media and Reagents *Page 1633*		
T 4661	**Non-sterile. Not pyrogen tested. White crystalline powder. Meets all specifications for T 1503 and all analytical and packaging requirements as per USP XXII. For biotechnology, diagnostics or manufacturing use.**	100 g	4200
RT		1 kg	23600
		5 kg	105900
		10 kg	188400
	Certificates of analysis are available with lot specific information.		
	Please inquire for commercial quantities.		
726-4	**1.5 M Solution**	100 ml	4200
HFC			
	TRIZMA ACETATE	25 g	4400
T 1258	(Tris[hydroxymethyl]aminomethane acetate)	100 g	12100
RT		250 g	30200
	Reagent Grade		
	[6850-28-8] $C_4H_{11}NO_3 \cdot C_2H_4O_2$ FW 181.2		
	TRIZMA BENZOATE	25 g	3400
T 0259	(Tris[hydroxymethyl]aminomethane benzoate)	100 g	8900
RT		250 g	17500
	Reagent Grade		
	[75221-97-5] $C_4H_{11}NO_3 \cdot C_7H_6O_2$ FW 243.3		
	TRIZMA CARBONATE	25 g	2400
T 9755	(Di[tris(hydroxymethyl)aminomethane] carbonate)	100 g	6400
HFC		250 g	12700
	Reagent Grade	1 kg	45600
	[68123-29-5] $(C_4H_{11}NO_3)_2 \cdot CH_2O_3$ FW 304.3	5 kg	204700
	TRIZMA CITRATE		
T 2910	**Monobasic**	50 ml	4400
RT	**2 Molar Solution**	250 ml	14600
	[108321-33-1]	500 ml	23000
T 2785	**Dibasic**	50 ml	3100
RT	**1 Molar Solution**	250 ml	10100
	[108321-34-2]	500 ml	16000

PRODUCT NUMBER			¥
	TRIZMA HYDROCHLORIDE		
	(Tris[hydroxymethyl]aminomethane hydrochloride)		
	pKa=8.1 at 25°C; useful pH range 7-9		
	[1185-53-1] $C_4H_{11}NO_3 \cdot HCl$ FW 157.6		
T 6666	**SigmaUltra**	50 g	7700
RT	**>99% (titration)**	250 g	27200
	pH (0.5 M in water, 20°C): 3.5-5.0	1 kg	81600
	Loss on drying (110°C): <0.2%		
	Residue on ignition (SO_4), 900°C): <0.2%		
	Solubility (0.5 M in water, 20°C): complete, colorless		
	Insoluble matter: passes filter test		
	Sulfate (SO_4): <0.005%	K: <0.005%	
	Al: <0.0005%	Li: <0.0005%	
	As: <0.0001%	Mg: <0.0005%	
	Ba: <0.0005%	Mn: <0.0005%	
	Bi: <0.0005%	Mo: <0.0005%	
	Ca: <0.001%	Na: <0.005%	
	Cd: <0.0005%	Ni: <0.0005%	
	Co: <0.0005%	Pb: <0.0005%	
	Cr: <0.0005%	Sr: <0.0005%	
	Cu: <0.0005%	Zn: <0.0005%	
	Fe: <0.0005%		
	A_{260}: <0.015; A_{280}: <0.010 (0.5 M in water)		
T 3253	**White crystalline powder**	100 g	6400
RT	**Reagent Grade**	250 g	9300
	Purity: 99% (min.) (potentiometric titration)	500 g	16000
		1 kg	27600
	$A^{40\%}_{280}$ **(1 cm path; vs. H_2O):** 0.05 (max.)	5 kg	109000
		6 × 1 kg	132100
	Water (Karl Fischer): 0.5% (max.)	10 kg	196100
	Heavy metals (as Pb): 0.0005% (max.)		
	A 40% (w/w) solution is clear and colorless.		
	See also: Tissue Culture Media and Reagents *Page 1633*		
	TRIZMAL™ BUFFER	50 ml	4200
870-2	**Solution of Trizma Maleate**		
2-8°C	0.02 M, pH 7.5 at 25°C; Autoclaved.		
	For use in the Determination of Plasma Heparin using Sigma Technical Bulletin No. 870.		
	See Diagnostic Kits and Reagents Section.		
	TRIZMA MALEATE	50 g	5400
T 3128	(Mono[tris(hydroxymethyl)-aminomethane] maleate)	100 g	8700
RT		250 g	17200
	Reagent Grade		
	[72200-76-1] $C_4H_{11}NO_3 \cdot C_4H_4O_4$ FW 237.2		
	TRIZMA NITRATE	25 g	3500
T 7132	(Tris[hydroxymethyl]aminomethane nitrate)	100 g	9200
-0°C		250 g	18100
	Reagent Grade		
	[41521-38-4] $C_4H_{11}NO_3 \cdot HNO_3$ FW 184.1		
	TRIZMA OXALATE	25 g	4000
T 7258	(Di[tris(hydroxymethyl)aminomethane] oxalate)	100 g	10900
RT		250 g	21700
	Reagent Grade		
	[108321-13-7] $C_{10}H_{24}N_2O_{10}$ FW 332.3		
	TRIZMA PHOSPHATE		
T 1758	(Mono[tris(hydroxymethyl)-aminomethane] phosphate)	25 g	5000
RT		100 g	12300
	Monobasic	250 g	24400
	Reagent Grade		
	See also: Trizma® Phosphate in Molecular Biology Products *Page 1551*		
	[6992-39-8] $C_4H_{11}NO_3 \cdot H_3PO_4$ FW 219.1		
T 4258	(Di[tris(hydroxymethyl)amino-methane] phosphate)	25 g	4700
RT		100 g	12800